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* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *
FILE 'HOME' ENTERED AT 12:24:44 ON 31 MAY 2005
=> file biosis caba caplus embase japio lifesci medline scisearch uspatfull
=> e frey joachim/au
E1            1     FREY JERGEN/AU
E2            1     FREY JIM/AU
E3          298 --> FREY JOACHIM/AU
E4            3     FREY JOANA/AU
E5            1     FREY JOCHEN/AU
E6            1     FREY JOERG/AU
E7            1     FREY JOHANN H/AU
E8           16     FREY JOHANN R/AU
E9           35     FREY JOHANN RUDOLF/AU
E10          10     FREY JOHANN WILHELM/AU
E11           4     FREY JOHN/AU
E12           1     FREY JOHN A/AU
=> s e3 and salmonicida
L1           17 "FREY JOACHIM"/AU AND SALMONICIDA
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2            8 DUP REM L1 (9 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 8 ANSWERS - CONTINUE? Y/(N):y

L2   ANSWER 1 OF 8  USPATFULL on STN
AN     2005:68524  USPATFULL
TI     Novel type III secretion pathway in Aeromonas   ***salmonicida***  , and
       uses therefor
IN       ***Frey, Joachim***  , Bern, SWITZERLAND
       Stuber, Katja, Ittigen, SWITZERLAND
       Thornton, Julian C., Victoria, CANADA
       Kuzyk, Michael A., Richmond, CANADA
       Burian, Jan, Victoria, CANADA
PA     Universitat Bern (non-U.S. corporation)
PI     US 2005058662      A1   20050317
AI     US 2004-813908     A1   20040326 (10)
RLI    Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.
       No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI   US 2000-248864P     20001115 (60)
DT     Utility
FS     APPLICATION
LREP   KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,
       97204
CLMN   Number of Claims: 15
ECL    Exemplary Claim: CLM-01-22
DRWN   5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Disclosed is a newly identified and characterized type III secretion
       system in Aeromonas   ***salmonicida***  . The invention also
       encompasses the use of components of the novel secretion system in
       immunoprotection against A.   ***salmonicida***   infection, as well as
       other diagnostic and therapeutic uses thereof.

L2   ANSWER 2 OF 8  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
     DUPLICATE 1
AN   2003:569435  BIOSIS
DN   PREV200300562558
TI   The ADP-ribosylating toxin, AexT, from Aeromonas   ***salmonicida***
     subsp.   ***salmonicida***   is translocated via a type III secretion
     pathway.
AU   Burr, Sarah E.; Stuber, Katja;   ***Frey, Joachim***   [Reprint Author]
CS   Institute of Veterinary Bacteriology, University of Berne,
     Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland
     joachim.frey@vbi.unibe.ch
SO   Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591.
     print.
     CODEN: JOBAAY. ISSN: 0021-9193.
DT   Article
LA   English
ED   Entered STN: 3 Dec 2003
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Last Updated on STN: 3 Dec 2003
AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen Aeromonas ***salmonicida*** subsp. ***salmonicida*** . The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have identified a further 12 open reading frames that possess high homology to genes encoding both structural and regulatory components of the Yersinia type III secretion apparatus. Using marker replacement mutagenesis of aopB, the A. ***salmonicida*** subsp. ***salmonicida*** homologue of yopB in Yersinia, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An acrV mutant of A. ***salmonicida*** subsp. ***salmonicida*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl2 concentrations as high as 10 mM. This acrV mutant is also unable to translocate AexT into the cytosol of fish cells, indicating AcrV is involved in the translocation process. Inactivation of either the aopB or acrV gene in A. ***salmonicida*** subsp. ***salmonicida*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant AcrV-His protein provides fish cells protection against the toxic effects of the bacterium.

L2 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2
AN 2003:720743 CAPLUS
DN 139:359791
TI Type III secretion genes in Aeromonas ***salmonicida*** subsp. ***salmonicida*** are located on a large thermolabile virulence plasmid
AU Stuber, Katja; Burr, Sarah E.; Braun, Martin; Wahli, Thomas; ***Frey,*** *** Joachim***
CS Institute of Veterinary Bacteriology, University of Bern, Bern, CH-3001, Switz.
SO Journal of Clinical Microbiology (2003), 41(8), 3854-3856
CODEN: JCMIDW; ISSN: 0095-1137
PB American Society for Microbiology
DT Journal
LA English
AB Type III secretion genes in Aeromonas ***salmonicida*** subsp. ***salmonicida*** are located on a large plasmid of approx. 140 kb, termed pASvirA. Cultivation of this organism at elevated temps. such as 25.degree.C can, however, result in loss of this plasmid. This is accompanied by an inability to secrete toxin AexTa and by a loss of virulence for cultured fish cells.
RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 3
AN 2004:164565 BIOSIS
DN PREV200400168104
TI Association of Type III secretion genes with virulence of Aeromonas ***salmonicida*** subsp. ***salmonicida*** .
AU Burr, Sarah E.; Wahli, Thomas; Segner, Helmut; Pugovkin, Dmitri; ***Frey, Joachim*** [Reprint Author]
CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, 3012, Berne, Switzerland
joachim.frey@vbi.unibe.ch
SO Diseases of Aquatic Organisms, (December 3 2003) Vol. 57, No. 1-2, pp. 167-171. print.
CODEN: DAOREO. ISSN: 0177-5103.
DT Article
LA English
ED Entered STN: 24 Mar 2004
Last Updated on STN: 24 Mar 2004
AB Aeromonas ***salmonicida*** subsp. ***salmonicida*** possesses a number of potential virulence factors, including a recently identified plasmid-encoded Type III secretion system. A number of field isolates of A. ***salmonicida*** subsp. ***salmonicida*** were examined for the presence of Type III secretion genes. Using in vitro experiments, it was found that field isolates containing such genes are cytotoxic to fish

cell lines, whereas those that lack these genes are not. Using a rainbow trout in vivo model, the virulence of a wild type A. ***salmonicida*** subsp. ***salmonicida*** strain (Strain JF2267), which possesses Type III secretion genes, was compared to that of a laboratory derivative of the same strain that has lost these genes. While Strain JF2267 was virulent towards rainbow trout, its derivative was not. The A. ***salmonicida*** subsp. ***salmonicida*** Type Strain ATCC 33658T, which also lacks Type III secretion genes, was also found to be avirulent by this challenge model. The findings from both the in vitro and in vivo experiments suggest that the presence of Type III secretion genes is associated with the virulence of this important fish pathogen.

L2 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391747 CAPLUS
DN 136:385049
TI Novel exoenzyme toxin of Aeromonas ***salmonicida*** and use for recombinant salmon furunculosis vaccine
IN ***Frey, Joachim*** ; Kuhnert, Peter; Braun, Martin; Thornton, Julian C.; Kuzik, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 2002023328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		

AB A novel protein toxin named Aeromonas ***salmonicida*** exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of A. ***salmonicida*** . Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against A. ***salmonicida*** infections.

L2 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN ***Frey, Joachim*** ; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA	2467309	AA	20020523	CA 2001-2467309	20011115
AU	2002014891	A5	20020527	AU 2002-14891	20011115
GB	2385854	A1	20030903	GB 2003-13912	20011115
NO	2003002217	A	20030709	NO 2003-2217	20030515
US	2005058662	A1	20050317	US 2004-813908	20040326
PRAI US	2000-248864P	P	20001115		
	WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L2 ANSWER 7 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 4
AN 2002:564959 BIOSIS
DN PREV200200564959
TI Evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida*** .
AU Burr, Sarah E.; Stuber, Katja; Wahli, Thomas; ***Frey, Joachim***
[Reprint author]
CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, CH-3012, Berne, Switzerland
joachim.frey@vbi.unibe.ch
SO Journal of Bacteriology, (November, 2002) Vol. 184, No. 21, pp. 5966-5970. print.
CODEN: JOBAAY. ISSN: 0021-9193.
DT Article
LA English
ED Entered STN: 7 Nov 2002
Last Updated on STN: 7 Nov 2002
AB Aeromonas ***salmonicida*** subsp. ***salmonicida*** , the etiological agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. ***salmonicida*** subsp. ***salmonicida*** yscV homologue, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. ***salmonicida*** subsp. ***salmonicida*** secretes proteins via a type in secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. ***salmonicida*** subsp. ***salmonicida*** strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphological changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.

L2 ANSWER 8 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 5
AN 2002:233045 BIOSIS
DN PREV200200233045
TI Characterization of an ADP-ribosyltransferase toxin (AexT) from Aeromonas ***salmonicida*** subsp. ***salmonicida*** .
AU Braun, Martin; Stuber, Katja; Schlatter, Yvonne; Wahli, Thomas; Kuhnert,

```
     Peter;   ***Frey, Joachim***   [Reprint author]
CS   Institute for Veterinary Bacteriology, University of Bern, Langgassstrasse
     122, CH-3012, Bern, Switzerland
     joachim.frey@vbi.unibe.ch
SO   Journal of Bacteriology, (April, 2002) Vol. 184, No. 7, pp. 1851-1858.
     print.
     CODEN: JOBAAY. ISSN: 0021-9193.
DT   Article
LA   English
ED   Entered STN: 10 Apr 2002
     Last Updated on STN: 10 Apr 2002
AB   An ADP-ribosylating toxin named Aeromonas   ***salmonicida***   exoenzyme
     T (AexT) in A.   ***salmonicida***   subsp.   ***salmonicida***   , the
     etiological agent of furunculosis in fish, was characterized. Gene aexT,
     encoding toxin AexT, was cloned and characterized by sequence analysis.
     AexT shows significant sequence similarity to the ExoS and ExoT exotoxins
     of Pseudomonas aeruginosa and to the YopE cytotoxin of different Yersinia
     species. The aexT gene was detected in all of the 12 A.
       ***salmonicida***   subsp.   ***salmonicida***   strains tested but was
     absent from all other Aeromonas species. Recombinant AexT produced in
     Escherichia coli possesses enzymatic ADP-ribosyltransferase activity.
     Monospecific polyclonal antibodies directed against purified recombinant
     AexT detected the toxin produced by A.   ***salmonicida***   subsp.
       ***salmonicida***   and cross-reacted with ExoS and ExoT of P. aeruginosa.
     AexT toxin could be detected in a wild type (wt) strain of A.
       ***salmonicida***   subsp.   ***salmonicida***   freshly isolated from a
     fish with furunculosis; however, its expression required contact with
     RTG-2 rainbow trout gonad cells. Under these conditions, the AexT protein
     was found to be intracellular or tightly cell associated. No AexT was
     found when A.   ***salmonicida***   subsp.   ***salmonicida***   was
     incubated in cell culture medium in the absence of RTG-2 cells. Upon
     infection with wt A.   ***salmonicida***   subsp.   ***salmonicida***   ,
     the fish gonad RTG-2 cells rapidly underwent significant morphological
     changes. These changes were demonstrated to constitute cell rounding,
     which accompanied induction of production of AexT and which led to cell
     lysis after extended incubation. An aexT mutant which was constructed
     from the wt strain with an insertionally inactivated aexT gene by allelic
     exchange had no toxic effect on RTG-2 cells and was devoid of AexT
     production. Hence AexT is directly involved in the toxicity of A.
       ***salmonicida***   subsp.   ***salmonicida***   for RTG-2 fish cells.


=> e stuber katja/au
E1          66     STUBER K/AU
E2           1     STUBER KATHARINA/AU
E3          16 --> STUBER KATJA/AU
E4           3     STUBER KENT/AU
E5           2     STUBER KERSTIN/AU
E6           2     STUBER KLAUS/AU
E7           3     STUBER KURT/AU
E8           3     STUBER L/AU
E9           2     STUBER LARRY M/AU
E10          1     STUBER LOVELL S/AU
E11        308     STUBER M/AU
E12          2     STUBER M I/AU

=> s e1-e3 and salmonicida
L3          29 ("STUBER K"/AU OR "STUBER KATHARINA"/AU OR "STUBER KATJA"/AU)
               AND SALMONICIDA

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4            6 DUP REM L3 (23 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 6 ANSWERS - CONTINUE? Y/(N):y


L4   ANSWER 1 OF 6  USPATFULL on STN
AN     2005:68524  USPATFULL
TI     Novel type III secretion pathway in Aeromonas   ***salmonicida***   , and
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uses therefor
IN Frey, Joachim, Bern, SWITZERLAND
Stuber, Katja , Ittigen, SWITZERLAND
Thornton, Julian C., Victoria, CANADA
Kuzyk, Michael A., Richmond, CANADA
Burian, Jan, Victoria, CANADA
PA Universitat Bern (non-U.S. corporation)
PI US 2005058662 A1 20050317
AI US 2004-813908 A1 20040326 (10)
RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser. No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI US 2000-248864P 20001115 (60)
DT Utility
FS APPLICATION
LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR, 97204
CLMN Number of Claims: 15
ECL Exemplary Claim: CLM-01-22
DRWN 5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.

L4 ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
AN 2003:569435 BIOSIS
DN PREV200300562558
TI The ADP-ribosylating toxin, AexT, from Aeromonas ***salmonicida*** subsp. ***salmonicida*** is translocated via a type III secretion pathway.
AU Burr, Sarah E.; ***Stuber, Katja*** ; Frey, Joachim [Reprint Author]
CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland
joachim.frey@vbi.unibe.ch
SO Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591. print.
CODEN: JOBAAY. ISSN: 0021-9193.
DT Article
LA English
ED Entered STN: 3 Dec 2003
Last Updated on STN: 3 Dec 2003
AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen Aeromonas ***salmonicida*** subsp. ***salmonicida*** . The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have identified a further 12 open reading frames that possess high homology to genes encoding both structural and regulatory components of the Yersinia type III secretion apparatus. Using marker replacement mutagenesis of aopB, the A. ***salmonicida*** subsp. ***salmonicida*** homologue of yopB in Yersinia, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An acrV mutant of A. ***salmonicida*** subsp. ***salmonicida*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl2 concentrations as high as 10 mM. This acrV mutant is also unable to translocate AexT into the cytosol of fish cells, indicating AcrV is involved in the translocation process. Inactivation of either the aopB or acrV gene in A. ***salmonicida*** subsp. ***salmonicida*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant AcrV-His protein provides fish cells protection against the toxic effects of the bacterium.

L4 ANSWER 3 OF 6 CABA COPYRIGHT 2005 CABI on STN DUPLICATE 2
AN 2003:177812 CABA
DN 20033152569

TI Type III secretion genes in Aeromonas ***salmonicida*** subsp. ***salmonicida*** are located on a large thermolabile virulence plasmid
AU ***Stuber, K.*** ; Burr, S. E.; Braun, M.; Wahli, T.; Frey, J.
CS Institute of Veterinary Bacteriology, University of Bern, Laenggassstrasse 122, Postfach, CH-3001 Bern, Switzerland. joachim.frey@vbi.unibe.ch
SO Journal of Clinical Microbiology, (2003) Vol. 41, No. 8, pp. 3854-3856. 6 ref.
Publisher: American Society for Microbiology (ASM). Washington
ISSN: 0095-1137
DOI: 10.1128/JCM.41.8.3854-3856.2003
CY United States
DT Journal
LA English
ED Entered STN: 20031107
Last Updated on STN: 20031107
AB Type III secretion genes in Aeromonas ***salmonicida*** subsp. ***salmonicida*** are located on a large plasmid of approximately 140 kb. Cultivation of this organism at elevated temperatures such as 25[deg]C can, however, result in loss of this plasmid. This is accompanied by a loss of virulence for cultured fish cells.

L4 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN Frey, Joachim; ***Stuber, Katja*** ; Thornton, Julian C.; Kuzyk, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
WO 2002040514	A3	20021227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L4 ANSWER 5 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 3
AN 2002:564959 BIOSIS
DN PREV200200564959
TI Evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida*** .
AU Burr, Sarah E.; ***Stuber, Katja*** ; Wahli, Thomas; Frey, Joachim [Reprint author]

CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, CH-3012, Berne, Switzerland joachim.frey@vbi.unibe.ch
SO Journal of Bacteriology, (November, 2002) Vol. 184, No. 21, pp. 5966-5970. print.
CODEN: JOBAAY. ISSN: 0021-9193.
DT Article
LA English
ED Entered STN: 7 Nov 2002
Last Updated on STN: 7 Nov 2002
AB Aeromonas ***salmonicida*** subsp. ***salmonicida*** , the etiological agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. ***salmonicida*** subsp. ***salmonicida*** yscV homologue, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. ***salmonicida*** subsp. ***salmonicida*** secretes proteins via a type in secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. ***salmonicida*** subsp. ***salmonicida*** strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphological changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.

L4 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 4
AN 2002:233045 BIOSIS
DN PREV200200233045
TI Characterization of an ADP-ribosyltransferase toxin (AexT) from Aeromonas ***salmonicida*** subsp. ***salmonicida*** .
AU Braun, Martin; ***Stuber, Katja*** ; Schlatter, Yvonne; Wahli, Thomas; Kuhnert, Peter; Frey, Joachim [Reprint author]
CS Institute for Veterinary Bacteriology, University of Bern, Langgassstrasse 122, CH-3012, Bern, Switzerland joachim.frey@vbi.unibe.ch
SO Journal of Bacteriology, (April, 2002) Vol. 184, No. 7, pp. 1851-1858. print.
CODEN: JOBAAY. ISSN: 0021-9193.
DT Article
LA English
ED Entered STN: 10 Apr 2002
Last Updated on STN: 10 Apr 2002
AB An ADP-ribosylating toxin named Aeromonas ***salmonicida*** exoenzyme T (AexT) in A. ***salmonicida*** subsp. ***salmonicida*** , the etiological agent of furunculosis in fish, was characterized. Gene aexT, encoding toxin AexT, was cloned and characterized by sequence analysis. AexT shows significant sequence similarity to the ExoS and ExoT exotoxins of Pseudomonas aeruginosa and to the YopE cytotoxin of different Yersinia species. The aexT gene was detected in all of the 12 A. ***salmonicida*** subsp. ***salmonicida*** strains tested but was absent from all other Aeromonas species. Recombinant AexT produced in Escherichia coli possesses enzymatic ADP-ribosyltransferase activity. Monospecific polyclonal antibodies directed against purified recombinant AexT detected the toxin produced by A. ***salmonicida*** subsp. ***salmonicida*** and cross-reacted with ExoS and ExoT of P. aeruginosa. AexT toxin could be detected in a wild type (wt) strain of A. ***salmonicida*** subsp. ***salmonicida*** freshly isolated from a fish with furunculosis; however, its expression required contact with RTG-2 rainbow trout gonad cells. Under these conditions, the AexT protein was found to be intracellular or tightly cell associated. No AexT was found when A. ***salmonicida*** subsp. ***salmonicida*** was incubated in cell culture medium in the absence of RTG-2 cells. Upon

infection with wt A. ***salmonicida*** subsp. ***salmonicida*** , the fish gonad RTG-2 cells rapidly underwent significant morphological changes. These changes were demonstrated to constitute cell rounding, which accompanied induction of production of AexT and which led to cell lysis after extended incubation. An aexT mutant which was constructed from the wt strain with an insertionally inactivated aexT gene by allelic exchange had no toxic effect on RTG-2 cells and was devoid of AexT production. Hence AexT is directly involved in the toxicity of A. ***salmonicida*** subsp. ***salmonicida*** for RTG-2 fish cells.

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=> e thornton julian c/au
E1            8     THORNTON JUDITH M/AU
E2            3     THORNTON JULIAN/AU
E3           22 --> THORNTON JULIAN C/AU
E4            1     THORNTON JULIAN CHARLES/AU
E5           17     THORNTON JULIE/AU
E6            1     THORNTON JULIE M/AU
E7            7     THORNTON JUSTIN/AU
E8            3     THORNTON JUSTIN A/AU
E9          166     THORNTON K/AU
E10           4     THORNTON K A/AU
E11           2     THORNTON K B/AU
E12          13     THORNTON K C/AU

=> s e2-e4 and salmonicida
L5           14 ("THORNTON JULIAN"/AU OR "THORNTON JULIAN C"/AU OR "THORNTON
                JULIAN CHARLES"/AU) AND SALMONICIDA

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6           12 DUP REM L5 (2 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 12 ANSWERS - CONTINUE? Y/(N):y


L6   ANSWER 1 OF 12  USPATFULL on STN
AN     2005:68524  USPATFULL
TI     Novel type III secretion pathway in Aeromonas   ***salmonicida***  , and
       uses therefor
IN     Frey, Joachim, Bern, SWITZERLAND
       Stuber, Katja, Ittigen, SWITZERLAND
           ***Thornton, Julian C.***  , Victoria, CANADA
       Kuzyk, Michael A., Richmond, CANADA
       Burian, Jan, Victoria, CANADA
PA     Universitat Bern (non-U.S. corporation)
PI     US 2005058662      A1   20050317
AI     US 2004-813908     A1   20040326 (10)
RLI    Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.
       No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI   US 2000-248864P     20001115 (60)
DT     Utility
FS     APPLICATION
LREP   KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,
       97204
CLMN   Number of Claims: 15
ECL    Exemplary Claim: CLM-01-22
DRWN   5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Disclosed is a newly identified and characterized type III secretion
       system in Aeromonas   ***salmonicida***  . The invention also
       encompasses the use of components of the novel secretion system in
       immunoprotection against A.   ***salmonicida***   infection, as well as
       other diagnostic and therapeutic uses thereof.

L6   ANSWER 2 OF 12  CAPLUS  COPYRIGHT 2005 ACS on STN
AN   2002:391747  CAPLUS
DN   136:385049
TI   Novel exoenzyme toxin of Aeromonas   ***salmonicida***   and use for
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recombinant salmon furunculosis vaccine
IN Frey, Joachim; Kuhnert, Peter; Braun, Martin; ***Thornton, Julian C.*** ; Kuzik, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 2002023328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		

AB A novel protein toxin named Aeromonas ***salmonicida*** exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of A. ***salmonicida*** . Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against A. ***salmonicida*** infections.

L6 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN Frey, Joachim; Stuber, Katja; ***Thornton, Julian C.*** ; Kuzyk, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467309	AA	20020523	CA 2001-2467309	20011115
	AU 2002014891	A5	20020527	AU 2002-14891	20011115
	GB 2385854	A1	20030903	GB 2003-13912	20011115
	NO 2003002217	A	20030709	NO 2003-2217	20030515
	US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses

the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L6 ANSWER 4 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 2001:333585 BIOSIS
DN PREV200100333585
TI Physiological and immunological effects of adjuvanted Aeromonas ***salmonicida*** vaccines on juvenile rainbow trout.
AU Ackerman, Paige A. [Reprint author]; Iwama, George K.; ***Thornton,*** *** Julian C.***
CS Faculty of Agricultural Sciences and Canadian Bacterial Diseases Network, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada ackerman@interchange.ubc.ca
SO Journal of Aquatic Animal Health, (June, 2000) Vol. 12, No. 2, pp. 157-164. print.
ISSN: 0899-7659.
DT Article
LA English
ED Entered STN: 18 Jul 2001
Last Updated on STN: 19 Feb 2002
AB The effects of injectable vaccines against Aeromonas ***salmonicida*** on oxygen consumption, growth, kidney lysozyme activity, and anti-A. ***salmonicida*** plasma antibody titers of juvenile rainbow trout Oncorhynchus mykiss were examined. The vaccines were A. ***salmonicida*** bacterin only, bacterin adjuvanted with levamisole, bacterin in emulsified oil, microencapsulated bacterin, microencapsulated bacterin with muramyl dipeptide, microencapsulated bacterin with beta-1,3-glucan, and microencapsulated bacterin with Vibrio anguillarum lipopolysaccharide (LPS). The greatest and broadest ranges of responses were caused by the microencapsulated bacterin with V. anguillarum LPS. Oxygen consumption rates and specific growth rates were significantly higher over the course of 1 month among fish treated with the LPS vaccine. These fish also maintained a higher anti-A. ***salmonicida*** plasma antibody titer and kidney lysozyme activity for a substantially longer period than fish receiving the other treatments.

L6 ANSWER 5 OF 12 USPATFULL on STN
AN 96:20896 USPATFULL
TI Attenuated strains of Aeromonas ***salmonicida*** useful as fish vaccines
IN ***Thornton, Julian C.*** , Brentwood Bay, Canada
Kay, William W., Victoria, Canada
PA University of Victoria, Victoria, Canada (non-U.S. corporation)
PI US 5498414 19960312
AI US 1992-957531 19921005 (7)
DT Utility
FS Granted
EXNAM Primary Examiner: Sidberry, Hazel F.; Assistant Examiner: Krsek-Staples, Julie
LREP Klarquist Sparkman Campbell Leigh & Whinston
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 1718
AB Novel attenuated strains of Aeromonas ***salmonicida*** are disclosed that are effective as live effective vaccines against furunculosis in fish. These vaccines may be administered by the immersion of fish in a solution of the vaccine. Methods of producing these strains and other strains having the identifying characteristics of these strains are also disclosed.

L6 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1996:409049 CAPLUS
DN 125:67377
TI The development of a live attenuated vaccine for the control of salmonid furunculosis (Aeromonas ***salmonicida*** , glucose catabolism, Oncorhynchus mykiss)

AU ***Thornton, Julian Charles***
CS Univ. of Victoria, Victoria, BC, Can.
SO (1994) 165 pp. Avail.: Univ. Microfilms Int., Order No. DANN03527
From: Diss. Abstr. Int., B 1996, 57(1), 105
DT Dissertation
LA English
AB Unavailable

L6 ANSWER 7 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 1994:86722 BIOSIS
DN PREV199497099722
TI Molecular biology of bacterial fish diseases.
AU ***Thornton, Julian C.*** ; Garduno, Rafael A.; Kay, William W.
CS Dep. Biochem. and Microbiol., Univ. Victoria, Victoria, BC V8W 3P6, Canada
SO Hochachka, P. W. [Editor]; Mommsen, T. P. [Editor]. (1993) pp. 159-189.
Biochemistry and Molecular Biology of Fishes; Molecular biology frontiers.
Publisher: Elsevier Science Publishers B.V., PO Box 211, Sara Burgerhartstraat 25, 1000 AE Amsterdam, Netherlands; Elsevier Science Publishing Co., Inc., P.O. Box 882, Madison Square Station, New York, New York 10159-2101, USA. Series: Biochemistry and Molecular Biology of Fishes.
ISBN: 0-444-81663-1.
DT Book
Book; (Book Chapter)
General Review; (Literature Review)
LA English
ED Entered STN: 5 Mar 1994
Last Updated on STN: 5 Mar 1994

L6 ANSWER 8 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
AN 1993:586018 BIOSIS
DN PREV199497005388
TI Novel antigens expressed by Aeromonas ***salmonicida*** growth in vivo.
AU ***Thornton, Julian C.*** ; Garduno, Rafael A.; Carlos, Steven J.; Kay, William W. [Reprint author]
CS Dep. Biochem. Microbiol., Canadian Bacterial Diseases Network, Univ. Victoria, VIC, BC V8W 3P6, Canada
SO Infection and Immunity, (1993) Vol. 61, No. 11, pp. 4582-4589.
CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 28 Dec 1993
Last Updated on STN: 28 Dec 1993
AB Virulent and avirulent Aeromonas ***salmonicida*** strains grown inside intraperitoneal implants in Rainbow trout (Oncorhynchus mykiss) were examined for unique antigen expression. Western blots (immunoblots), performed with immune rabbit serum raised against in vivo-grown cells, revealed several unique antigens. With the exception of lipopolysaccharide (LPS), these novel antigens were destroyed after proteinase K treatment. The majority of these antigens were not induced in vitro in response to either iron limitation or anaerobiosis. In addition, electron microscopy demonstrated the presence of a putative capsule on in vivo-grown cells. Purification and fractionation of this carbohydrate material from cells grown in carbon-rich synthetic media resulted in the isolation and separation of an antigenically distinct LPS not seen with cells grown in standard media. Antiserum raised against in vivo-grown cells recognized both this LPS and the typical LPS of A. ***salmonicida*** apparent in in vitro-grown cells. Antiserum raised against in vitro-grown cells recognized only the LPS expressed in vitro. Antiserum directed against in vivo-grown cells was approximately 10 times more sensitive than serum directed against in vitro-grown cells in detecting A. ***salmonicida*** in infected fish kidney tissue.

L6 ANSWER 9 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
AN 1993:479464 BIOSIS
DN PREV199396113064
TI Aeromonas ***salmonicida*** grown in vivo.
AU Garduno, Rafael A.; ***Thornton, Julian C.*** ; Kay, William W.

[Reprint author]
CS Canadian Bacterial Disease Network, Univ. Victoria, P.O. Box 3055, Victoria, British Columbia V8W 3P6, Canada
SO Infection and Immunity, (1993) Vol. 61, No. 9, pp. 3854-3862. CODEN: INFIBR. ISSN: 0019-9567.
DT Article
LA English
ED Entered STN: 22 Oct 1993
Last Updated on STN: 22 Oct 1993
AB The virulent fish pathogen Aeromonas ***salmonicida*** was rapidly killed in vivo when restricted inside a diffusion chamber implanted intraperitoneally in rainbow trout. After a period of regrowth, the survivors had acquired resistance to host-mediated bacteriolysis, phagocytosis, and oxidative killing, properties which were subsequently lost by growth in vitro. Resistance to bacteriolysis and phagocytosis was associated with a newly acquired capsular layer revealed by acidic polysaccharide staining and electron microscopy. This capsular layer shielded the underlying, regular surface array (S-layer) from immunogold labeling with a primary antibody to the S-layer protein. Resistance to oxidative killing was mediated by a mechanism not associated with the presence of the capsular layer. An attenuated vaccine strain of A. ***salmonicida*** grown in vivo failed to express the capsular layer. Consequently, the in vivo-grown cells of this attenuated strain remained as sensitive to bacteriolysis, and as avidly adherent to macrophages, as the in vitro-grown cells. The importance of these new virulence determinants and their relation to the known virulence factors of A. ***salmonicida*** are discussed.

L6 ANSWER 10 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
AN 1994:77914 BIOSIS
DN PREV199497090914
TI Fate of the fish pathogen Aeromonas ***salmonicida*** in the peritoneal cavity of rainbow trout.
AU Garduno, Rafael A.; ***Thornton, Julian C.*** ; Kay, William W. [Reprint author]
CS Dep. Biochem. and Microbiol., Univ. Victoria, Petch Bldg., P.O. Box 3055, Victoria, BC V8W 3P6, Canada
SO Canadian Journal of Microbiology, (1993) Vol. 39, No. 11, pp. 1051-1058. CODEN: CJMIAZ. ISSN: 0008-4166.
DT Article
LA English
ED Entered STN: 22 Feb 1994
Last Updated on STN: 22 Feb 1994
AB A model was developed to study the fate of the fish pathogen Aeromonas ***salmonicida*** in vivo, inside a specialized intraperitoneal chamber implanted in rainbow trout, Oncorhynchus mykiss. Although normally recalcitrant to lytic agents in vitro, owing to the presence of its regular surface array (S layer), A. ***salmonicida*** was rapidly killed in the peritoneal cavity by a host-derived, soluble lytic activity present in peritoneal fluid. Peritoneal fluid was also found to kill other bacteria and lyse various types of erythrocytes, but was particularly lytic to A. ***salmonicida*** . Intraperitoneal survival of injected (free) A. ***salmonicida*** cells was several orders of magnitude higher than survival of implanted (restrained) cells. Injected free cells could evade the lytic activity of peritoneal fluid because they readily spread, initiating lethal infections. One evasion strategy was envisioned to be the penetration of peritoneal and (or) tissue macrophages. In spite of the killing mechanisms of these phagocytic cells, A. ***salmonicida*** was still able to survive and even replicate inside head kidney macrophages, thereby supporting the notion of A. ***salmonicida*** as a facultatively intracellular pathogen. Intraperitoneal chambers in rainbow trout may constitute a valuable experimental tool for studying the in vivo fate of A. ***salmonicida*** , and perhaps of other fish pathogens as well.

L6 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1994:553182 CAPLUS
DN 121:153182
TI Does the S-layer of Aeromonas ***salmonicida*** exist in more than one functional organizational state?

AU Garduno, Rafael A.; ***Thornton, Julian C.*** ; Kay, William W.
CS Department Biochemistry and Microbiology and Canadian Bacterial Disease Network, University Victoria, Victoria, BC, Can.
SO NATO ASI Series, Series A: Life Sciences (1993), 252(ADVANCES IN BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 285-8
CODEN: NALSDJ; ISSN: 0258-1213
DT Journal
LA English
AB The S-layer of Aeromonas ***salmonicida*** posseses significant flexibility and plasticity. The functional relevance of one of these novel structural patters (big squares pattern) was studied here. The big squares pattern was effective in protecting cells from complement-mediated bacteriolysis and oxidative killing; this pattern also mediated a very efficient assocn. of Aeromonas with macrophage and the hemin anal Congo Red. Host-mediated structural changes in the S-layer may be involved in pathogenesis.

L6 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN
AN 1994:529448 CAPLUS
DN 121:129448
TI Structure - function aspects of the Aeromonas ***salmonicida*** S-layer
AU Kay, William W.; ***Thornton, Julian C.*** ; Garduno, Raphael A.
CS Department Biochemistry and Microbiology, University Victoria, Victoria, BC, Can.
SO NATO ASI Series, Series A: Life Sciences (1993), 252(ADVANCES IN BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 151-8
CODEN: NALSDJ; ISSN: 0258-1213
DT Journal; General Review
LA English
AB A review with 22 refs.

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E8            1     KUZYK PAUL R/AU
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                ALLAN"/AU) AND SALMONICIDA

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L8             3 DUP REM L7 (1 DUPLICATE REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y
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L8 ANSWER 1 OF 3 USPATFULL on STN
AN 2005:68524 USPATFULL
TI Novel type III secretion pathway in Aeromonas ***salmonicida*** , and uses therefor
IN Frey, Joachim, Bern, SWITZERLAND
Stuber, Katja, Ittigen, SWITZERLAND
Thornton, Julian C., Victoria, CANADA
Kuzyk, Michael A. , Richmond, CANADA
Burian, Jan, Victoria, CANADA
PA Universitat Bern (non-U.S. corporation)
PI US 2005058662 A1 20050317
AI US 2004-813908 A1 20040326 (10)

RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser. No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI US 2000-248864P 20001115 (60)
DT Utility
FS APPLICATION
LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR, 97204
CLMN Number of Claims: 15
ECL Exemplary Claim: CLM-01-22
DRWN 5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; ***Kuzyk, Michael*** *** A.*** ; Burian, Jan
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
WO 2002040514	A3	20021227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L8 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
AN 1998:98617 BIOSIS
DN PREV199800098617
TI Structural and physiological determinants of resistance of Aeromonas ***salmonicida*** to reactive radicals.
AU Garduno, Rafael A.; ***Kuzyk, Michael A.*** ; Kay, William W. [Reprint author]
CS Dep. Biochemistry Microbiol., Canadian Bacterial Disease Network, Univ. Victoria, P.O. Box 3055, Victoria, BC V8W 3P6, Canada
SO Canadian Journal of Microbiology, (Nov., 1997) Vol. 43, No. 11, pp.

1044-1053. print.
CODEN: CJMIAZ. ISSN: 0008-4166.
DT Article
LA English
ED Entered STN: 25 Feb 1998
Last Updated on STN: 25 Feb 1998
AB The facultative intracellular pathogen Aeromonas ***salmonicida*** survives and replicates in macrophages, a virulence trait presumed to be associated with its ability to resist reactive radicals. The mechanisms used by A. ***salmonicida*** to resist reactive radicals in vitro were shown to have both structural and physiological determinants. The sensitivity of A. ***salmonicida*** to exogenous H2O2, superoxide, and nitrogen radicals, as well as endogenous oxygen radicals, differed depending on growth conditions, cell surface structure, and preexposure to sublethal doses of radicals. Whereas sensitivities to exogenous oxygen radicals did not correlate with basal levels of catalase or Fe-superoxide dismutase, under similar culture conditions S-layer positive cells were more resistant to oxygen radicals than S-layer mutants. S-layer mutants recovered resistance when physically reconstituted with S-layer sheets. Hemin-coated S-layers, while protective against nitrogen radicals, sensitized A. ***salmonicida*** to H2O2. Sublethal concentrations of H2O2 or superoxide induced a highly protective response characterized by de novo synthesis of both catalase and Mn-superoxide dismutase. It is proposed that for A. ***salmonicida*** the constitutive S-layer provides a first line of defense and the inducible catalase and Mn-superoxide dismutase provide a powerful second line of defense against macrophage-mediated killing via reactive oxygen species.

```
=> e burian jan/au
E1            3     BURIAN J A/AU
E2            1     BURIAN JAMES E/AU
E3           42 --> BURIAN JAN/AU
E4            1     BURIAN JAROSLAW/AU
E5            1     BURIAN JEEMUSU TOMUPUSON/AU
E6            2     BURIAN JIEEMUSU EDOWAAZU/AU
E7            1     BURIAN JIEEMUSU TETSUDOMAASHIYU/AU
E8            1     BURIAN JIEEMUSU TOOMASU/AU
E9            1     BURIAN JIEI BIIRIYUU/AU
E10           1     BURIAN JIEI SURIIBAN/AU
E11          11     BURIAN JIRI/AU
E12           1     BURIAN JIYOJIFU ROBO/AU

=> s e3 and salmonicida
L9            3 "BURIAN JAN"/AU AND SALMONICIDA

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10           3 DUP REM L9 (0 DUPLICATES REMOVED)

=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L10  ANSWER 1 OF 3  USPATFULL on STN
AN     2005:68524  USPATFULL
TI     Novel type III secretion pathway in Aeromonas ***salmonicida*** , and
       uses therefor
IN     Frey, Joachim, Bern, SWITZERLAND
       Stuber, Katja, Ittigen, SWITZERLAND
       Thornton, Julian C., Victoria, CANADA
       Kuzyk, Michael A., Richmond, CANADA
           ***Burian, Jan*** , Victoria, CANADA
PA     Universitat Bern (non-U.S. corporation)
PI     US 2005058662      A1   20050317
AI     US 2004-813908     A1   20040326 (10)
RLI    Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.
       No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI   US 2000-248864P     20001115 (60)
DT     Utility
FS     APPLICATION
LREP   KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,
```

97204
CLMN Number of Claims: 15
ECL Exemplary Claim: CLM-01-22
DRWN 5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.

L10 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391747 CAPLUS
DN 136:385049
TI Novel exoenzyme toxin of Aeromonas ***salmonicida*** and use for recombinant salmon furunculosis vaccine
IN Frey, Joachim; Kuhnert, Peter; Braun, Martin; Thornton, Julian C.; Kuzik, Michael A.; ***Burian, Jan***
PA Switz.
SO PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 2002023328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		

AB A novel protein toxin named Aeromonas ***salmonicida*** exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of A. ***salmonicida*** . Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against A. ***salmonicida*** infections.

L10 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; ***Burian, Jan***
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				

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            GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
            LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
            PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
            UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
        RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
            CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
            BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
    CA 2467309          AA    20020523    CA 2001-2467309        20011115
    AU 2002014891       A5    20020527    AU 2002-14891          20011115
    GB 2385854          A1    20030903    GB 2003-13912          20011115
    NO 2003002217       A     20030709    NO 2003-2217           20030515
    US 2005058662       A1    20050317    US 2004-813908         20040326
PRAI US 2000-248864P    P     20001115
    WO 2001-CA1589      W     20011115
AB  Disclosed is a newly identified and characterized type III secretion
    system in Aeromonas   ***salmonicida***   . The invention also encompasses
    the use of components of the novel secretion system in immunoprotection
    against A.   ***salmonicida***   infection, as well as other diagnostic
    and therapeutic uses thereof.  The components of type III secretion system
    of Aeromonas   ***salmonicida***   useful as fish vaccines are B cell
    epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR,
    AcrG, AcrV, and AcrH.


=> s salmonicida
L11       7273 SALMONICIDA

=> s l11 and acr?
L12        258 L11 AND ACR?

=>

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13         193 DUP REM L12 (65 DUPLICATES REMOVED)

=> s l13 and ((acr1)or(acr2)or(acr3)or(acr4)or(acrd)or(acrr)or(acrg)or(acrv)or(acrh))
L14         27 L13 AND ((ACR1) OR(ACR2) OR(ACR3) OR(ACR4) OR(ACRD) OR(ACRR)
               OR(ACRG) OR(ACRV) OR(ACRH))

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 27 ANSWERS - CONTINUE? Y/(N):y

L14  ANSWER 1 OF 27  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
AN   2003:569435  BIOSIS
DN   PREV200300562558
TI   The ADP-ribosylating toxin, AexT, from Aeromonas   ***salmonicida***
     subsp.   ***salmonicida***   is translocated via a type III secretion
     pathway.
AU   Burr, Sarah E.; Stuber, Katja; Frey, Joachim [Reprint Author]
CS   Institute of Veterinary Bacteriology, University of Berne,
     Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland
     joachim.frey@vbi.unibe.ch
SO   Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591.
     print.
     CODEN: JOBAAY. ISSN: 0021-9193.
DT   Article
LA   English
ED   Entered STN: 3 Dec 2003
     Last Updated on STN: 3 Dec 2003
AB   AexT is an extracellular ADP ribosyltransferase produced by the fish
     pathogen Aeromonas   ***salmonicida***   subsp.   ***salmonicida***   .
     The protein is secreted by the bacterium via a recently identified type
     III secretion system.  In this study, we have identified a further 12 open
     reading frames that possess high homology to genes encoding both
     structural and regulatory components of the Yersinia type III secretion
     apparatus.  Using marker replacement mutagenesis of aopB, the A.
       ***salmonicida***   subsp.   ***salmonicida***   homologue of yopB in
     Yersinia, we demonstrate that the bacterium translocates the AexT toxin
     directly into the cytosol of cultured fish cells via this type III
     secretion pathway.  An   ***acrV***   mutant of A.   ***salmonicida***
```

subsp. ***salmonicida*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl2 concentrations as high as 10 mM. This ***acrV*** mutant is also unable to translocate AexT into the cytosol of fish cells, indicating ***AcrV*** is involved in the translocation process. Inactivation of either the aopB or ***acrV*** gene in A. ***salmonicida*** subsp. ***salmonicida*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant ***AcrV*** -His protein provides fish cells protection against the toxic effects of the bacterium.

TI The ADP-ribosylating toxin, AexT, from Aeromonas ***salmonicida*** subsp. ***salmonicida*** is translocated via a type III secretion pathway.

AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen Aeromonas ***salmonicida*** subsp. ***salmonicida*** . The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have. . . both structural and regulatory components of the Yersinia type III secretion apparatus. Using marker replacement mutagenesis of aopB, the A. ***salmonicida*** subsp. ***salmonicida*** homologue of yopB in Yersinia, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An ***acrV*** mutant of A. ***salmonicida*** subsp. ***salmonicida*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl2 concentrations as high as 10 mM. This ***acrV*** mutant is also unable to translocate AexT into the cytosol of fish cells, indicating ***AcrV*** is involved in the translocation process. Inactivation of either the aopB or ***acrV*** gene in A. ***salmonicida*** subsp. ***salmonicida*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant ***AcrV*** -His protein provides fish cells protection against the toxic effects of the bacterium.

IT . . . Concepts
Infection; Molecular Genetics (Biochemistry and Molecular Biophysics); Toxicology

IT Parts, Structures, & Systems of Organisms
cytosol

IT Diseases
Aeromonas ***salmonicida*** ***salmonicida*** infection: bacterial disease

IT Chemicals & Biochemicals
AexT: ADP ribosyltransferase, toxin; calcium chloride; open reading frame; recombinant ***AcrV*** -His protein

ORGN Classifier
Aeromonadaceae 06701

Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms

Organism Name
Aeromonas ***salmonicida*** ***salmonicida*** (subspecies): pathogen

Taxa Notes
Bacteria, Eubacteria, Microorganisms

ORGN Classifier
Pisces 85200

Super Taxa
Vertebrata; Chordata; Animalia

Organism Name
fish (common):. . .

GEN Aeromonas ***salmonicida*** ***salmonicida*** ***acrV*** gene (Aeromonadaceae); Aeromonas ***salmonicida*** ***salmonicida*** aopB gene (Aeromonadaceae)

L14 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:806113 CAPLUS
DN 138:86215
TI Evidence for a type III secretion system in Aeromonas ***salmonicida***

subsp. ***salmonicida***
AU Burr, Sarah E.; Stuber, Katja; Wahli, Thomas; Frey, Joachim
CS Institute of Veterinary Bacteriology and Centre for Fish and Wildlife Health, University of Berne, Bern, CH-3012, Switz.
SO Journal of Bacteriology (2002), 184(21), 5966-5970
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB Aeromonas ***salmonicida*** subsp. ***salmonicida*** , the etiol. agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. ***salmonicida*** subsp. ***salmonicida*** yscV homolog, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. ***salmonicida*** subsp. ***salmonicida*** secretes proteins via a type III secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. ***salmonicida*** subsp. ***salmonicida*** strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphol. changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.
RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI Evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***
AB Aeromonas ***salmonicida*** subsp. ***salmonicida*** , the etiol. agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. ***salmonicida*** subsp. ***salmonicida*** yscV homolog, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. ***salmonicida*** subsp. ***salmonicida*** secretes proteins via a type III secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. ***salmonicida*** subsp. ***salmonicida*** strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphol. changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***acr1*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***acr2*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)
IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***acrG*** ; evidence for a type III secretion system in Aeromonas

salmonicida subsp. ***salmonicida***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(***acrR*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(aopN; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(ascN; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(ascV; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(ascX; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Aeromonas ***salmonicida*** ***salmonicida***
DNA sequences
Protein sequences
Secretion (process)
Virulence (microbial)
(evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT ADP ribosylation factor
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ***acr1*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ***acr2*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ***acrG*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ***acrR*** ; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene aopN; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ascN; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(gene ascV; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ascX; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Proteins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene ascY; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT Operon
(virA; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT 484256-36-2 484256-37-3 484256-38-4 484256-39-5 484256-40-8
484256-41-9 484256-42-0 484256-43-1 484256-44-2
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

IT 465948-29-2
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; evidence for a type III secretion system in Aeromonas ***salmonicida*** subsp. ***salmonicida***)

L14 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2002:391746 CAPLUS
DN 136:400588
TI T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines
IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; Burian, Jan
PA Switz.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
WO 2002040514	A3	20021227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. ***Acr1*** , ***Acr2*** , ***Acr3*** , ***Acr4*** , ***AcrD*** , ***AcrR*** , ***AcrG*** , ***AcrV*** , and ***AcrH*** .

TI T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines

AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas ***salmonicida*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. ***Acr1*** , ***Acr2*** , ***Acr3*** , ***Acr4*** , ***AcrD*** , ***AcrR*** , ***AcrG*** , ***AcrV*** , and ***AcrH*** .

ST Aeromonas ***salmonicida*** ***AcrD*** ***AcrV*** epitope vaccine

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***Acr1*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***Acr2*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***Acr3*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***Acr4*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***AcrG*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***AcrH*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***AcrR*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***AcrV*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(AexT; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Aeromonas ***salmonicida***
Antiserums
Blood serum
Culture media
Drug delivery systems

Epitopes
Fish
Genetic vectors
Oncorhynchus mykiss
Plasmids
Spraying
Susceptibility (genetic)
Vaccines
(T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Antibodies and Immunoglobulins
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Gene, microbial
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Oligonucleotides
Probes (nucleic acid)
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Gene, microbial
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***acrD*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Gene, microbial
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(***acrV*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Immunostimulants
(adjuvants; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Gene, microbial
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aexT; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Diagnosis
(agents; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT B cell (lymphocyte)
T cell (lymphocyte)
(epitope; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Animal tissue
(fish; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Microorganism
(host cell; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(immersion; T or B cell epitopes of ***AcrV*** or ***AcrD*** of

type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(injections, i.m.; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(injections, i.p.; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Matrix media
(insol.; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(intracellular; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(intradermal; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Drug delivery systems
(oral; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Mutagenesis
(site-directed, addn.; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Mutagenesis
(site-directed, deletion; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Mutagenesis
(site-directed, substitution; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT ADP ribosylation factor
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(toxin; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT Proteins
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(type III secretion app.; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT 58319-92-9, Exoenzyme T
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(Aeromonas ***salmonicida*** ; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT 429112-86-7 429112-88-9 429113-04-2 429113-21-3 429113-22-4 429113-31-5 429113-34-8 429113-35-9 429113-36-0
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT 429113-37-1
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; T or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

IT 429115-35-5 429115-36-6 429115-37-7 429115-38-8 429115-39-9 429115-40-2 429115-41-3 429115-42-4 429115-43-5

RL: PRP (Properties)
(unclaimed sequence; t or B cell epitopes of ***AcrV*** or ***AcrD*** of type III secretion pathway in Aeromonas ***salmonicida*** for use as vaccines)

L14 ANSWER 4 OF 27 USPATFULL on STN
AN 2005:68524 USPATFULL
TI Novel type III secretion pathway in Aeromonas ***salmonicida*** , and uses therefor
IN Frey, Joachim, Bern, SWITZERLAND
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PA Universitat Bern (non-U.S. corporation)
PI US 2005058662 A1 20050317
AI US 2004-813908 A1 20040326 (10)
RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser. No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI US 2000-248864P 20001115 (60)
DT Utility
FS APPLICATION
LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR, 97204
CLMN Number of Claims: 15
ECL Exemplary Claim: CLM-01-22
DRWN 5 Drawing Page(s)
LN.CNT 1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.
TI Novel type III secretion pathway in Aeromonas ***salmonicida*** , and uses therefor
AB Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.
SUMM . . . relates to bacterial secretion systems, and in particular to a newly identified and characterized type III secretion system in Aeromonas ***salmonicida*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against A. ***salmonicida*** infection, as well as other diagnostic and therapeutic uses thereof.
SUMM [0003] Aeromonas ***salmonicida*** , a Gram-negative, facultatively anaerobic, non-motile, rod shaped bacterium, growing at temperatures around 20.degree. C., is the etiological agent of furunculoses. . .
SUMM [0005] Knowledge of the mechanisms of pathogenicity of A. ***salmonicida*** , and in particular of the main virulence factors involved, is essential in the development of efficient strategies to prevent outbreaks of furunculoses caused by A. ***salmonicida*** . Currently, several potential virulence factors of A. ***salmonicida*** have been reported, including a surface-layer protein (Chu et al., 1991), the hemolysins ASH1, ASH3, ASH4 (Hirono and Aoki, 1993),. . . is unclear and many of them seem not to play a primary role in virulence. This was demonstrated by A. ***salmonicida*** strains with deletion mutants of the GCAT and aspA genes which had no influence on virulence of the strains in. . .
SUMM . . . ADP-ribosylating toxin named AexT (Aeromonas exoenzyme T) encoded by the gene aexT was identified in a virulent strain of A. ***salmonicida*** . A. ***salmonicida*** strains that were propagated for several passages on culture medium had lost expression of AexT, but still retained the aexT. . .
SUMM [0007] Based on these observations, we used broad range gene probes to identify in A. ***salmonicida*** a novel type III secretion system by means of the gene ***acrD*** (Aeromonas calcium response D) encoding a transmembrane spanning protein. The ***acrD*** gene has a high similarity to IcrD, a protein of the Yersinia sp. which is an inner

membrane protein of the type III secretion apparatus in Yersinia sp. The ***acrD*** gene is flanked by further typical type III secretion genes which were designated ***acr1*** , ***acr2*** , ***acr3*** , ***acr4*** , ***acrD*** , ***acrR*** , ***acrG*** , ***acrV*** , and ***acrH*** , and which show significant similarity to pcr1, pcr2, pcr3, pcr4, pcrD, pcrR, pcrG, pcrV, and pcrH of Pseudomonas aeruginosa and. . . the respective bacterium, including the regulation of the low calcium response (LCR) and chaperon functions. The genes isolated from A. ***salmonicida*** belong to the analogue of the virA operon, which is central in the type III secretion pathway of many Gram-negative. . .

SUMM [0008] We have also determined that the type III secretion system in A. ***salmonicida*** is located on a 84 kb plasmid which is rapidly lost upon growth in culture medium. Biosynthesis of ***AcrV*** in A. ***salmonicida*** , the analogue to LcrV in Yersinia, requires as a trigger either low Ca.sup.2+ conditions or contact with fish cells. Upon infection with A. ***salmonicida*** expressing ***AcrV*** , the cultured cells undergo significant morphological changes. Cultures derived from originally virulent A. ***salmonicida*** strains, which had lost the type III secretion genes including ***AcrV*** , lost virulence as they did not affect rainbow trout gonad cells morphologically after infection. Concomitantly to loss of the type III secretion genes, these cultures lost the expression of the aexT gene which specifies the ADP-ribosylating toxin of A. ***salmonicida*** .

SUMM [0009] Rainbow trout gonad cells infected with the virulent A. ***salmonicida*** and incubated in antiserum directed against recombinant ***AcrV*** -His protein could be protected from the toxic effect and showed only weak morphological changes. ***AcrV*** , which belongs to the type III secretion proteins is a determinative factor involved in virulence mechanisms of A. ***salmonicida*** , and is expected to provide new insights into basic mechanisms of pathogenicity of bacterial species. The components of the type III secretion system of A. ***salmonicida*** may be used as antigens for the development of sub-unit vaccines against infection of fish by A. ***salmonicida*** .

SUMM . . . invention comprises an isolated 5.7 kb nucleic acid segment (SEQ ID NO:10) containing the type III secretion genes of A. ***salmonicida*** . In another embodiment, the invention comprises a nucleic acid segment that encodes protein having the amino acid sequence of SEQ. . .

SUMM [0011] In a further embodiment, the invention relates to the use of ***AcrV*** as an immunogen, and to the use of ***AcrV*** in a recombinant or traditional vaccine to reduce the incidence of infection by A. ***salmonicida*** .

SUMM [0012] In another embodiment, the invention provides a means of diagnosing A. ***salmonicida*** , or other bacteria found to contain ***AcrV*** homologues, by the detection of the ***AcrV*** protein or the homologous proteins.

DRWD [0013] FIG. 1 is a genetic map of the type III secretion genes found in A. ***salmonicida*** . Boxes with arrowheads indicate open reading frames (ORFs). The size of the different genes (in kilobases) is shown by the scale bar. A restriction map containing restriction enzymes SacI, PstI, NotI, BamHI, and SalI is shown. Abbreviation used: ***acr*** , Aeromonas calcium response.

DRWD [0014] FIG. 2 is a segregation curve of A. ***salmonicida*** JF2267. An A. ***salmonicida*** JF2267 LB-culture was first incubated 21/2 hrs at 19.degree. C. and then at 22.degree. C. for 7 hrs. Colony-blotting was. . .

DRWD [0015] FIG. 3 shows a pulsed-field gel electrophoresis of A. ***salmonicida*** strain JF2267, and strain JF2397. (Lane 1) JF2267, undigested. (Lane 2) JF2397, undigested. (Lane 3) JF2267 digested with NotI. (Lane. . . Low Range PFG Marker (New England Biolabs). The white arrows indicate the bands that hybridized on Southern blots with the ***acrD*** gene probe.

DRWD [0016] FIG. 4 shows infection of fish cells with A. ***salmonicida*** ATCC 33658.sup.T, JF2267, and JF2397. RTG-2 cells infected with JF2267 (A), ATCC 33658.sup.T (B), JF2397 (C), and pure PBS (D). RTG-2 cells infected with JF2267 and monospecific polyclonal antibodies against ***AcrV*** were protected (E), whereas RTG-2 cells infected with JF2267 and anti- ***AcrV*** preserum were not. Pictures were taken 24 hrs after infection, respectively 21 hrs after the protection assay under a phase. . .

DRWD [0017] FIG. 5 shows low Ca.sup.2+ response induced ***AcrV*** expression in A. ***salmonicida*** JF2267. The picture shows an immunoblot reacted with specific rabbit anti- ***AcrV*** antiserum. Strains ATCC 33658.sup.T (lane 2), JF2267 (lane 3) and JF2397 (lane 4) were grown in Ca.sup.2+ depleted medium, harvested by centrifugation and analyzed on 15% SDS PAGE followed by immunoblotting. Lane 1 contains purified recombinant ***AcrV*** -His protein as a control.

DETD [0018] A 5.7 kb segment containing type III secretion genes of A. ***salmonicida*** that were cloned and sequenced correspond to the pcr locus (Pseudomonas calcium response) of Pseudomonas aeruginosa (Frank, 1997; Yahr et. . . Gough et al., 1992; Michiels and Cornelis, 1991). The most conserved gene at this locus was revealed to be the ***acrD*** gene encoding the ***AcrD*** protein, which showed 82% identical aa to the transmembrane spanning core proteins LcrD of the injectisome of the Y. enterocolitica. . . aeruginosa type III secretion apparatus (Yahr et al., 1997b; Plano et al., 1991). Due to this high similarity, we conclude ***AcrD*** to have the analogous functions in the injectisome of the A. ***salmonicida*** type III secretion pathway.

DETD [0019] The least conserved protein encoded on the cloned and analyzed segment is ***AcrV*** , which shows only 35% identical aa to PcrV of P. aeruginosa and 37% identity to LcrV of Y. enterocolitica. The main role of LcrV and PcrV, and accordingly also of ***AcrV*** , is assumed to be involved in sensing the bacterium-host interactions (Sawa et al., 1999; Bergman et al., 1991). We therefore interpret the significantly higher dissimilarity between ***AcrV*** and LcrV or PcrV, compared to the other gene products of the type III secretion locus (Table 2), to be due to the host specificity which seems to be determined by ***AcrV*** , LcrV or PcrV.

DETD [0020] Our analyses revealed the A. ***salmonicida*** type III secretion genes to be located on a plasmid of 84 kb. The plasmid was shown to be lost. . . in particular after a slight raise in growth temperature. Concomitant to the loss of the type III genes in A. ***salmonicida*** , we detected the loss in virulence of the strain as measured by the infection of RTG-2 fish cell cultures, as. . . the loss of production of ADP-ribosylating toxin aexT in supernatants and bacterial cell pellets of low Ca.sup.2+ response induced A. ***salmonicida*** cultures. It is also noted that AexT biosynthesis induced by contact of A. ***salmonicida*** with RTG-2 fish cells disappeared in those strains or subcultures that had lost the type III secretion genes. Expression of. . .

DETD . . . III secretion mechanism was also indicated by the presence of a consensus sequence upstream the aexT toxin gene in A. ***salmonicida*** , which shows full homology to the binding site of a transcriptional activator, known in P. aeruginosa as ExsA, which is involved in type III dependent gene expression (Frank, 1997). The expression of aexT in A. ***salmonicida*** is thus dependent on a functional type III secretion mechanism. The lack of production of AexT as detected in the type strain of A. ***salmonicida*** ATCC 33658.sup.T as well as in the strain JF2397 which was derived from an originally virulent A. ***salmonicida*** strain, JF2267, in spite of the presence of a functional aexT gene, must therefore be due to the loss of. . .

DETD [0022] The ***AcrV*** protein of the novel type III secretion pathway of A. ***salmonicida*** plays an important role in pathogenesis by its role as a sensor and regulator of the system, as shown in. . . efficiently protected mice against challenge with Y. pestis (Leary et al., 1995). Our results showed that antibodies directed against recombinant ***AcrV*** , the analogous protein to LcrV, protected fish RTG-2 cells from damage caused by virulent A. ***salmonicida*** strain JF2267 and demonstrated that the ***AcrV*** plays an important role in type III secretion pathway mediated virulence of A. ***salmonicida*** .

DETD [0023] The newly found type III secretion pathway plays a central role in pathogenicity of A. ***salmonicida*** via the secretion and direct injection of the ADP-ribosylating toxin AexT into the target cells. Loss of the type III. . . due to the instability of a kb plasmid under culture conditions. Furthermore, loss of type III secretion genes such as ***acrD*** and ***acrV*** abolished expression of the aexT gene, and led to loss of virulence of A. ***salmonicida*** . As shown, surface exposed gene products of this

type III secretion pathway, in particular ***AcrV*** , are potent candidates for new vaccines for the immune prophylaxis of fish against furunculosis.

DETD [0027] A. ***salmonicida*** strains are listed in Table 1. A. ***salmonicida*** type strain ATCC 33658.sup.T was purchased from the American Type Culture Collection. A. ***salmonicida*** strain JF2267 was freshly isolated from an arctic char (Savelinus alpinus) showing typical symptoms of furunculoses. A. ***salmonicida*** strain JF2397 was derived from strain JF2267 by repeated single colony isolations after each of nine passages propagated on LB agar medium at 22.degree. C. for two days each passage. A. ***salmonicida*** strains were routinely cultured on blood agar plates (Trypticase soy agar supplemented with 0.1% CaCl.sub.2 and 5% sheep blood) at. . .

DETD [0028] Liquid cultures of A. ***salmonicida*** were made by inoculation of Tripticase soy broth (TSB) (2.75 g/100 ml Tripticase soy broth without Dextrose (BBL.RTM. 11774, Becton. . .

DETD [0031] Genomic DNA of A. ***salmonicida*** was extracted by the guanidium hydrochloride method (Pitcher et al., 1989). A partial gene library of, A. ***salmonicida*** JF2267 was constructed by cloning agarose gel purified SacI-SalI digested fragments of 4 to 6 kb size into vector pBluescriptII-SK.sup.-. . . blot (Ausubel et al., 1999) using digoxigenin (DIG)-labeled DNA probes as described previously (Braun et al., 1999). Plasmids from A. ***salmonicida*** were purified using the method of Birnboim and Doly (Birnboim and Doly, 1979).

DETD [0032] To construct a genomic library from A. ***salmonicida*** JF2267, 0.1 .mu.g of DNA partially digested with Sau3a was ligated to ZapExpress BamHI prepared arms (Pharmacia, Uppsala, Sweden) and. . .

DETD . . . and NBRF databases were performed using the programs BLASTN, BLASTX and BLASTP (Altschul et al., 1990). Potentially antigenic segments of ***AcrV*** were determined using the software ProtScale (http://www.expasy.ch/cgi-bin/protscale.pl (Bairoch et al., 1995) and the software Coils output (htpp://www.ch.embnet.org/software/COILS_form.html) (Lupas et al.,. . .

DETD [0039] Curing of Type III Secretion Genes from A. ***salmonicida*** :

DETD [0040] In order to study the segregation of the type III secretion genes in A. ***salmonicida*** strain JF2267, the strain was inoculated in LB-broth at a density of A.sub.600=0.08 and incubated 21/2 hrs at 19.degree. C.. . .

DETD [0042] The bacterial strains A. ***salmonicida*** JF 2267 and JF2397 were grown on LB agar for one day at room temperature. Then bacterial suspensions in 10. . .

DETD [0046] Expression and Purification of His-Tailed Fusion Protein ***AcrV*** :

DETD [0047] Oligonucleotide primers used to amplify the whole ***acrV*** gene are given in Table 2. The PCR reactions were carried out as described above with the exception of using. . . DNA polymerase (Expand Long Template PCR System kit, Roche Diagnostics) instead of Taq DNA polymerase and genomic DNA of A. ***salmonicida*** JF2267. The PCR products were purified by using the High Puree PCR Product Purification Kit (Roche Diagnostics) as described by the manufacturer's protocol. Then the ***acrV*** PCR product was cloned into pGEM-T vector (Promega, Madison, Wis. USA), having 3'-T overhangs at the insertion sites, as described. . .

DETD [0048] Production of Monospecific Rabbit Anti- ***AcrV*** Antibodies and Immunoblot Analyses:

DETD [0049] Monospecific, polyclonal antibodies directed against ***AcrV*** were obtained by immunizing rabbits subcutaneous with 80 .mu.g of recombinant polyhistidine-tailed ***AcrV*** protein in 200 .mu.l PN buffer and 150 .mu.l NaCl (0.85%) mixed with 350 .mu.l Freund's complete adjuvant (Difco Laboratories,. . .

DETD [0050] Infection of Fish Cell Cultures with A. ***salmonicida*** :

DETD . . . and 4 mio cells were seeded into a 25 cm.sup.2 tissue culture flask. Monolayered RTG-2 cells were infected with A. ***salmonicida*** cells resuspended in phosphate buffered saline (PBS) pH 7.4 at a multiplicity of infection of 20:1 or 2:1 (bacteria/fish cells).. . .

DETD [0052] Protection Assay Using Rabbit Antiserum ***AcrV*** :

DETD . . . cells were seeded into 24 well culture plates (1.9 cm.sup.2) (Techno plastic products AG, Trasadingen, Switzerland). Rabbit antiserum directed against ***AcrV*** as well as control preserum were decomplemented for 30 min at 56.degree. C. A fresh culture of A. ***salmonicida*** (at end exponential growth phase) was washed and

resuspended in PBS pH 7.4 and mixed with either preserum or anti ***AcrV*** antiserum at a ratio of 1:1, 1:10, 1:100, 1:1000 or 1:10,000. Bacteria were incubated with the serum at 18.degree. C.. . .

DETD . . . immunoblotting, Western-blots were blocked with 1% milk buffer for at least one hour and then incubated with the rabbit antiserum ***AcrV*** (1:2000) or with the rabbit preserum (1:1000) in milk buffer overnight at 4.degree. C. The membranes were then washed thoroughly. . .

DETD [0056] Cloning and Sequence Analysis of the virA Locus of a Type III Pathway of A. ***salmonicida*** :

DETD [0057] Analysis of A. ***salmonicida*** strain JF2267 with an array of broad range probes for detection of type III secretion pathways revealed a strong signal. . . type III secretion pathway of Yersinia pests and Pseudomonas aeruginosa. In analogy to the Y. pestis genes, we named them ***acr1*** , ***acr2*** , ***acr3*** , ***acr4*** , and ***acrD*** (Aeromonas calcium response (FIG. 1)). They are located on a single operon followed by a transcription termination signal similar to. . . 1996; Iriarte and Cornelis, 1999; Plano et al., 1991; Cornelis, 1998; Yahr et al., 1997a). The similarities of the genes ***acr1*** , ***acr2*** , ***acr3*** , ***acr4*** and ***acrD*** with the analogues in Y. enteroclitica and in P. aeruginosa are given in Table 2. Downstream lcrD we identified a locus with a canonical promoter sequence followed by further genes named ***acrR*** , ***acrG*** , and ***acrV*** on a separate operon (FIG. 1) according to the corresponding genes in Y. pestis (Table 3) (Barve and Straley, 1990; Skrzypek and Straley, 1993; Nilles et al., 1998). The ORF of the putative ***acrV*** gene seemed to be incomplete on the 4.8 kb SacI-SalI fragment of pJFFIVB638, and represented only the 5'-half of the gene. The remaining part of ***acrV*** and part of ***acrH*** located downstream of ***acrV*** were cloned separately from the .lambda. phage gene library of A. ***salmonicida*** as an overlapping clone which was obtained by screening the gene library using a gene probe for the 5'-half of ***acrV*** which was produced by PCR with primers ***AcrV*** -L and ***AcrV*** -R (Table 2). The resulting plasmid based on vector pBK-CMV was designated pJFFIVB832. From this plasmid, a 0.9 kb SalI fragment containing the 3' end of ***acrV*** and part of the downstream gene ***acrH*** was subcloned on pBluescriptII-SK and designated pJFFIVB828.

DETD [0058] Instability of the Genes Belonging to the Type III Pathway in A. ***salmonicida*** :

DETD [0059] When we analyzed the different A. ***salmonicida*** strains with a specific probe for ***acrD*** , we discovered by using Southern blot hybridization that the ***acrD*** gene was present only in strain JF2267 but not in the derivative strain JF2397 which had undergone nine passages of subsequent single colony cloning isolation. Additionally, the type strain of A. ***salmonicida*** , ATCC 33658.sup.T, did not show a signal with the ***acrD*** probe. However, several A. ***salmonicida*** strains that were freshly isolated from salmon and trout with furunculoses did contain ***acrD*** (Table 4). These results indicate that the type III secretion pathway of A. ***salmonicida*** may be lost easily. In order to get an estimate on the loss of the type III secretion genes, we have analyzed the kinetics of disappearance of ***acrD*** after a shift of growth temperature of strain JF2267 from 19.degree. C. to 22.degree. C. Colony hybridization with the ***acrV*** probe revealed that in a fresh culture of strain JF2267, the ***acrD*** gene was present in all cells grown at 19.degree. C. After the shift to 22.degree. C., ***acrD*** was still present for further 51/2 hrs, following which it was lost very rapidly within less than 1 hr (FIG. 2). Taking into account the generation time of 2 h for A. ***salmonicida*** under the given growth conditions, the ***acrD*** gene was lost within two generations. To analyze the loss of ***acrD*** further, undigested and NotI digested genomic DNA of A. ***salmonicida*** strain JF2267 and of the ***acrD*** deficient derivative strain JF2397 were submitted to pulse field gel electrophoresis (PFGE) and subsequent Southern blot hybridization with the ***acrD*** probe. PFGE analyses of total undigested DNA revealed the presence of two large plasmids in strain JF2267 while in strain. . . compared to JF2267 as the sole detectable difference (FIG. 3). Southern-blot hybridization of the DNA on this gels with the ***acrD*** probe confirmed the larger plasmid and the 84 kb NotI

fragment of strain JF 2267 to contain ***acrD*** gene. Neither the remaining large plasmid in JF2397 nor any of its NotI fragments hybridized with the ***AcrV*** probe. This indicates that the type III secretion genes, or at least the virA operon thereof, are located on a. . .

DETD [0060] Presence of ***acrD*** in A. ***salmonicida*** Strains:

DETD [0061] In order to assess the presence of the ***acrD*** gene in various A. ***salmonicida*** strains, DNA samples extracted from A. ***salmonicida*** Type strain ATCC33658 and various field strains isolated from salmon or char were digested with restriction enzymes SalI and SacI, separated by 0.7% agarose gel electrophoresis, blotted onto nylon membranes and hybridized with the ***acrD*** gene probe. The Southern blot revealed the presense of the ***acrD*** gene on a 4.8 kb fragment in all strains except in the type strain ATCC33658, the laboratory strain JF2396 which was used for the type III secretion genes, and A. ***salmonicida*** strain MT44 known to be a virulent for trout. One field strain, # 24, showed a very weak hybridization signal indicating that the culture contains ***acrD*** only in a minor population of the cells (Table 1).

DETD [0062] Infection of RTG-2 Fish Cells and Protection of Cell Damage with Anti- ***AcrV*** Antiserum:

DETD [0063] Freshly cultured A. ***salmonicida*** strain JF2267 was used to infect RTG-2 cells. After 24 hrs of incubation the fish cells were rounded up and also detached from the plastic support (FIG. 4A). In contrast cells infected with A. ***salmonicida*** type strain ATCC 33658.sup.T or strain JF2397 (FIGS. 4B and C), both known to be devoid of ***acrD*** and ***acrV*** , showed no morphological changes at all in spite of a massive multiplication of the bacteria in the cultures. RTG-2 fish cells which were incubated with PBS buffer as control showed no morphological changes like the cells infected with the ***acrD*** and ***acrV*** deficient strains JF2397 or ATCC 33658.sup.T (FIG. 4D).

DETD [0064] In order to study further the role of the newly detected type III secretion pathway in virulence of A. ***salmonicida*** , we incubated strain JF 2267 with monospecific polyclonal anti- ***AcrV*** antibodies prior to infection of RTG-2 fish cell cultures. When RTG-2 fish cells were infected with strain JF2267 that was incubated with rabbit anti- ***AcrV*** antibodies diluted 1:1 or 1:10, the characteristic morphological changes of the cells were reduced, significantly affecting only 20% of the. . . with JF 2267 that was pretreated with serum from the same rabbit taken before immunization (FIG. 4F). Titration of the anti- ***AcrV*** serum showed that protection of about 50% of the RTG-2 cells could still be reached with a serum dilution of. . .

DETD [0065] Expression of ***AcrV*** in A. ***salmonicida*** :

DETD [0066] The expression of ***AcrV*** in A. ***salmonicida*** strain JF2267 was assessed by immunoblots using ***AcrV*** -His antibodies. When A. ***salmonicida*** was grown under standard culture conditions in TSB medium, no ***AcrV*** protein could be detected from total cells nor from culture supernatant of strain JF 2267, nor in the control of. . . Ca.sup.2+ response by chelating free Ca.sup.2+ ions in the growth medium by the addition of 10 mM NTA, we detected ***AcrV*** with anti- ***AcrV*** antibodies in the pellet of JF2267 as a protein of about 37 kDa (FIG. 5) but not in strains JF2397 and ATCC33658.sup.T, which are both devoid of the ***AcrV*** gene (FIG. 5). No ***AcrV*** protein could be detected in the supernatants of cultures from strains JF2267, JF2396 and ATCC33658.sup.T, grown in Ca.sup.2+ depleted medium.

DETD . . . Ca.sup.2+ ions) and then put in contact with RTG-2 cells at a ratio 2:1 (bacteria: cells) for 30 minutes, the ***AcrV*** protein could be monitored on immunoblots reacting with anti- ***AcrV*** , similar to cultures from Ca.sup.2+ depleted medium.

DETD [0068] Recombinant ***AcrV*** Vaccine Trial

DETD . . . M., Kay, W. W. and Trust, T. J.: Structure of the tetragonal surface virulence array protein and gene of Aeromonas ***salmonicida*** . J.Biol.Chem. 266 (1991) 15258-15265.

DETD [0091] Hirono, I. and Aoki, T.: Cloning and characterization of three hemolysin genes from Aeromonas ***salmonicida*** . Microb.Pathog. 15 (1993) 269-282.

DETD . . . K. and Ellis, A. E.: Glycerophospholipid:cholesterol acyltransferase complexed with lipopolysaccharide (LPS) is a major

lethal exotoxin and cytolysin of Aeromonas ***salmonicida*** : LPS stabilizes and enhances toxicity of the enzyme. J.Bacteriol. 172 (1990) 5382-5393.

DETD [0112] Thornton, J. C., Garduno, R. A., Carlos, S. J. and Kay, W. W.: Novel antigens expressed by Aeromonas ***salmonicida*** grown in vivo. Infect.Immun. 61 (1993) 4582-4589.

DETD [0113] Titball, R. W. and Munn, C. B.: The purification and some properties of H-lysin from Aeromonas ***salmonicida***. . J.Gen.Microbiol. 131 (1985) 1603-1609.

DETD . . . P. W., Landon, M. and Coleman, G.: The cloning and nucleotide sequence of the serine protease gene (aspA) of Aeromonas ***salmonicida*** ssp. ***salmonicida*** . FEMS Microbiol.Lett. 78 (1992) 65-71.

DETD [0118] Recombinant ***AcrV*** Vaccine Trial

DETD [0121] 1. The ***AcrV*** vaccine was formulated using recombinant, Histidine-tagged ***AcrV*** resuspended in 10 mM phosphate buffer, pH 7.0, to 112.5 .mu.g/mL. Four parts of this protein solution were mixed with one part oil adjuvant for a final ***AcrV*** concentration of 90 .mu.g/mL. The dose for testing was 0.1 mL, or 9 .mu.g/fish.

DETD [0128] At least 50 fish are vaccinated 0.1 mL of ***AcrV*** vaccine via intra-peritoneal (IP) injection, or 0.2 mL of the commercial vaccine MultiVacc4. At the same time a group of. . .

DETD . . . 350-degree days post vaccination 50 fish per group were challenged by IP injection with a pre-determined concentration of virulent Aeromonas ***salmonicida*** . The dosage depends on the source of the fish and the water temperature (this is determined empirically immediately prior to. . .

DETD [0133] Results

Group	% Mortality	RPS
PBS	82	--
AcrV	49	40
MultiVacc4	30	63

DETD [0134] 1. There was a strong challenge with 82% control mortalities.

TABLE 1

A. ***salmonicida*** strains used in this study and presence of ***acrD***

strain	origin	***acrD*** .sup.a)
ATCC33658	American Type Culture Collection, Type strain	-
JF2267	Char (Savelinus alpinus), Switzerland	+
JF2396	Laboratory strain, derivative of JF2267	-
CC-23	Salmon, Norway. . . blot hybridization .sup.b)	very weak hybridization signal indicating that only a minor part of the population of the culture contains the ***acrD*** gene

DETD . . .

TABLE 2

Oligonucleotide primers

Name	Seguence.sup.a 5' to 3'	Position.sup.b	Annealing temp. .degree. C.
AslcrD-L.sup.c	GCCCGTTTTGCCTATCAA	1159-1176	60
AslcrD-R.sup.c	GCGCCGATATCGGTACCC	2028-2011	60
AcrV -L.sup.c	TTCGTCGGCTGGCTTGATGT	4144-4163	58
AcrV -R.sup.c	GAACTCGCCCCCTTCCATAA	4734-4715	58
AsacrVt-L.sup.d	gggaattcGATGAGCACAATCCCTGACTAC	4104-4125	57

AsacrVt-Rd atgcggccgcAAATTGCGCCAAGAATGTCG 5188-5169 57

AsacrVN'-R.sup.d tcgcggccgcACCCTTTACGCTGATTGTC 4555-4537 57

AsacrVC'-L.sup.d cggaattcGTTGCGGGATGAGCTGGCAG 4554-4573 57

AsacrVC'-R.sup.d. . . 57

.sup.aLowercase letters indicate nucleotides added to create restriction enzyme recognition sites (underlined) for cloning.
.sup.bBased on nucleotide sequence of A. ***salmonicida*** JF2267
.sup.cPrimer used for gene probe preparation
.sup.dPrimer used for amplification of gene ***acrV*** , ***acrV*** -N, and ***acrV*** -C respectively
DETD [0136]
TABLE 3

A. ***salmonicida*** type III proteins compared to analogues In P. aeruginosa and in V. entercolitica.

Protein in A. ***salmonicida***	Analogue in P. aeruginosa	Similarlty/ identity.sup.a)	Genbank access. nr.	Analogue in Y. enterocolitica	Genbank access. nr.	Proposed function
Acr1	Pcr1	80/60	AF019150	TyeA	83/69	AF102990	part of the translocation-control apparatus, required for selective translocation of Yops
Acr2	Pcr2	63/44	AF019150	SycN	77/62	AF102990	chaperone forYopN
Acr3	Pcr3	62/47	AF019150	YscX	69/54	AF102990	part of the type III secretion apparatus, secretion of Yop
Acr4	Pcr4	66/55	AF019150	YscY	64/52	AP102990	part of the type III secretion apparatus, secretion of Yop
AcrD	PcrD	90/82	AF019150	LcrD	90/82	X87771	inner membrane spanning protein of type III secretion
AcrR	PcrR	68/58	AF019150	LcrR	71/58	AF102990	
AcrG	PcrG	63/46	AF010149	LcrG	64/42	AF102990	regulation of low calcium response
AcrV	PcrV	50/35	AF010149	LcrV	53/37	X96797	regulation of low calcium response, sensor suppression of TNFa and Interferon a, protective antigen
AcrH	PcrH	78/65	AF010149	LcrH (SycD)	79/58	AF102990	regulation ot low calcium response, chaperon for YopD, secretion

.sup.a)given as % of similar/identical. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 2
LENGTH: 123
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 2
Met Asn Trp Ile Glu Pro Leu Leu Val Gln Phe Cys Gln Asp Leu Gly
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 3
LENGTH: 121
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 3
Met Ser Arg Ile Thr Ala Ala His Ile Gly Ile Glu Gln Leu Ser Ala
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 4

LENGTH: 116
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 4
Met Thr Met Val Leu Thr Ser Gln Gln Gln Asp Ala Leu Leu Leu Thr
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 5
LENGTH: 705
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 5
Met Asn Gln Arg Thr Leu Glu Leu Leu Arg Arg Ile Gly Glu Arg Lys
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 6
LENGTH: 93
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 6
Met Leu Val Arg Arg Glu Gly Glu Arg Ala Gly Leu Ala Asn Pro Phe
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 7
LENGTH: 94
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 7
Met Lys Gln Pro Arg Phe Ala Asp His Ser Glu Thr Ile Ser Gln Ala
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 8
LENGTH: 361
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 8
Met Ser Thr Ile Pro Asp Tyr Asn Thr Asn Pro Gly Ala Phe Val Gly
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 9
LENGTH: 159
TYPE: PRT
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 9
Met Gln Thr Asp Thr Thr Leu Thr Pro Glu Tyr Glu Ala Glu Leu Glu
1 5 10. . .
DETD SEQUENCE CHARACTERISTICS:
SEQ ID NO: 10
LENGTH: 5678
TYPE: DNA
ORGANISM: Aeromonas ***salmonicida***
SEQUENCE: 10
gagctcaagc ggctgatccg cctgctgccg gtggagctgt tcagtgaaga ggagcagcgc 60
cagaatctgt tgcagtgctg tcagggtgcg ctcgataacg ccatcgagcg ggaagaggat 120
gagttgtctg gagagtcgtc atgaactgga ttgaacccct gctggtgcag. . .
CLM What is claimed is:

23. An isolated polypeptide comprising at least one epitope or epitopic region of a selected one of the class ***Acr1*** ; ***Acr2*** ; ***Acr3*** ; ***Acr4*** ; ***AcrD*** ; ***AcrR*** ; ***AcrG*** ; ***AcrV*** ; and ***AcrH*** .

29. A method for reducing the susceptibility of fish to infection by a virulent strain of A. ***salmonicida*** comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of a composition comprising an immunogenic amount of at least one epitope or epitopic region of ***AcrV*** , any other protein of the A. ***salmonicida*** Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof.

. . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. ***salmonicida*** Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .

. . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. ***salmonicida*** Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .

. . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. ***salmonicida*** Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .

33. A method for reducing the susceptibility of fish to infection by a virulent strain of A. ***salmonicida*** comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of an immunogenic amount of a composition comprising the ***acrV*** gene, the gene of any other protein of the A. ***salmonicida*** Type III secretion apparatus, homologues, fragments, or synthetic oligonucleotides derived thereof.

34. A method for reducing the susceptibility of fish to infection by a virulent strain of A. ***salmonicida*** comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of an immunogenic amount of a. . .

35. A therapeutic method for the protection of fish from the toxic effect of a virulent strain of A. ***salmonicida*** comprising the use of antiserum directed against ***AcrV*** , variants or fragments thereof, or synthesized peptides thereof.

36. The method of claim 35 wherein the antiserum is directed against recombinant ***AcrV*** .

L14 ANSWER 5 OF 27 USPATFULL on STN
AN 2004:7465 USPATFULL
TI Poroplasts
IN Surber, Mark W., Coronado, CA, UNITED STATES
Giacalone, Matthew, San Diego, CA, UNITED STATES
PI US 2004005700 A1 20040108
AI US 2002-157339 A1 20020528 (10)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 18
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18539
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.
DRWD [0313] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DRWD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
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in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DRWD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DRWD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DRWD [0471] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DRWD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DRWD [0581] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DRWD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DRWD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 6 OF 27 USPATFULL on STN

AN 2003:330124 USPATFULL

TI Minicell-based screening for compounds and proteins that modulate the activity of signalling proteins

IN Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES

PI US 2003232335 A1 20031218

AI US 2002-157317 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18564

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

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DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

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DETD [0575] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 7 OF 27 USPATFULL on STN
AN 2003:318700 USPATFULL
TI Antibodies to native conformations of membrane proteins
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
PI US 2003224444 A1 20031204
AI US 2002-157491 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18559
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0313] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0464] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0569] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .
DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .
DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino

acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 8 OF 27 USPATFULL on STN
AN 2003:318625 USPATFULL
TI Reverse screening and target identification with minicells
IN Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES
PI US 2003224369 A1 20031204
AI US 2002-157171 A1 20020528 (10)
PRAI US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18610
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0467] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0576] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 9 OF 27 USPATFULL on STN

AN 2003:312291 USPATFULL

TI Minicell-based bioremediation

IN Segall, Anca M., San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003219888 A1 20031127

AI US 2002-157418 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18632

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0347] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic

Acid Res. Mol. Biol. 66:107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0488] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng LW, Schneewind O. Type III machines of Gram-negative bacteria: delivering the. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0581] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 10 OF 27 USPATFULL on STN

AN 2003:311814 USPATFULL

TI Methods of making pharmaceutical compositions with minicells

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003219408 A1 20031127

AI US 2002-157320 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18632

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);

angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 11 OF 27 USPATFULL on STN
AN 2003:300375 USPATFULL
TI Minicell-based delivery agents
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
PI US 2003211599 A1 20031113
AI US 2002-157106 A1 20020528 (10)
RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING
PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18671

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 12 OF 27 USPATFULL on STN
AN 2003:299865 USPATFULL
TI Minicell-based selective absorption

IN Berkley, Neil, San Diego, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PI US 2003211086 A1 20031113
AI US 2002-157073 A1 20020528 (10)
PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18553
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0313] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5 S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0470] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0578] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .
DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can

also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 13 OF 27 USPATFULL on STN
AN 2003:294815 USPATFULL
TI Pharmaceutical compositions with minicells
IN Berkley, Neil, San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PI US 2003207833 A1 20031106
AI US 2002-156811 A1 20020528 (10)
PRAI US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18585
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0468] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0577] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 14 OF 27 USPATFULL on STN

AN 2003:288723 USPATFULL

TI Conjugated minicells

IN Surber, Mark W., Coronado, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003203481 A1 20031030

AI US 2002-157213 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18551

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene

(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0467] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0576] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 15 OF 27 USPATFULL on STN

AN 2003:288653 USPATFULL

TI Methods of minicell-based delivery

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003203411 A1 20031030

AI US 2002-156792 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18582

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** :

identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 16 OF 27 USPATFULL on STN
AN 2003:288179 USPATFULL
TI Minicell-based diagnostics
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
PI US 2003202937 A1 20031030
AI US 2002-157178 A1 20020528 (10)
PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)

LN.CNT 18527

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0314] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRi and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0471] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0580] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 17 OF 27 USPATFULL on STN

AN 2003:282746 USPATFULL
TI Membrane to membrane delivery
IN Surber, Mark W., Coronado, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES
PI US 2003199089 A1 20031023
AI US 2002-157318 A1 20020528 (10)
PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18530
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access

through lipid membranes, which are generally hydrophobic.. . .
DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .
DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 18 OF 27 USPATFULL on STN
AN 2003:282745 USPATFULL
TI Minicell-based gene therapy
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
PI US 2003199088 A1 20031023
AI US 2002-156902 A1 20020528 (10)
PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 15300
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By

"semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0502] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0611] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 19 OF 27 USPATFULL on STN
AN 2003:282662 USPATFULL
TI Solid supports with minicells
IN Sabbadini, Roger, Lakeside, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES
PI US 2003199005 A1 20031023
AI US 2002-157166 A1 20020528 (10)
RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING
PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18494
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful fór applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein

traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0469] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0578] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 20 OF 27 USPATFULL on STN

AN 2003:282653 USPATFULL

TI Minicell libraries

IN Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003198996 A1 20031023

AI US 2002-157147 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2001-293566P 20010524 (60)
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18482

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0314] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of

Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0472] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0582] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 21 OF 27 USPATFULL on STN
AN 2003:282652 USPATFULL
TI Forward screening with minicells
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
Gerhart, William, La Mesa, CA, UNITED STATES
PI US 2003198995 A1 20031023
AI US 2002-156831 A1 20020528 (10)
RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18533
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0314] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRl homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0471] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind 0. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0580] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .
DETD [0669] XIII. Minicell-based Delevery of Biologically ***Acrive*** Agents
DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of

non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 22 OF 27 USPATFULL on STN

AN 2003:276773 USPATFULL

TI Minicell compositions and methods

IN Surber, Mark W., Coronado, CA, UNITED STATES
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003194798 A1 20031016

AI US 2002-154951 A1 20020524 (10)

PRAI US 2001-293566P 20010524 (60)
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18583

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DRWD [0345] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DRWD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DRWD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DRWD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66: 107-157);the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DRWD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DRWD [0504] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope

(for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DRWD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DRWD [0613] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DRWD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DRWD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 23 OF 27 USPATFULL on STN

AN 2003:276689 USPATFULL

TI Minicell-based transformation

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003194714 A1 20031016

AI US 2002-157299 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18595

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0348] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5 S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene

(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0505] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0613] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 24 OF 27 USPATFULL on STN

AN 2003:271080 USPATFULL

TI Minicell-based rational drug design

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003190683 A1 20031009

AI US 2002-157302 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 15

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18539

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate

N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 25 OF 27 USPATFULL on STN
AN 2003:270998 USPATFULL
TI Target display on minicells
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Surber, Mark W., Coronada, CA, UNITED STATES
PI US 2003190601 A1 20031009
AI US 2002-157096 A1 20020528 (10)
RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING
PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)

LN.CNT 18581
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0349] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0504] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0609] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .
DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .
DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyomithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 26 OF 27 USPATFULL on STN
AN 2003:238122 USPATFULL

TI Minicell-based transfection
IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
PI US 2003166279 A1 20030904
AI US 2002-157391 A1 20020528 (10)
RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING
PRAI US 2002-359843P 20020225 (60)
US 2001-293566P 20010524 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
CLMN Number of Claims: 18
ECL Exemplary Claim: 1
DRWN 2 Drawing Page(s)
LN.CNT 18548
AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.
DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .
DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).
DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .
DETD [0469] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .
DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .
DETD [0578] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access

through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 27 OF 27 USPATFULL on STN

AN 2003:237942 USPATFULL

TI Minicells comprising membrane proteins

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES
Surber, Mark W., Coronado, CA, UNITED STATES
Berkley, Neil, San Diego, CA, UNITED STATES
Segall, Anca M., San Diego, CA, UNITED STATES
Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003166099 A1 20030904

AI US 2002-157305 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18580

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnositic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to ***acrR*** (Ma, D., et al. 1996. The local repressor ***AcrR*** plays a modulating role in the regulation of ***acrAB*** genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas ***salmonicida*** (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas ***salmonicida*** : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across*** the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and ***across***

the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged ***across*** the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0502] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins ***across*** the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds ***across*** membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0611] A "channel protein" is a protein that facilitates the diffusion of molecules/ions ***across*** lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents ***across*** the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyldextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- ***acrylamide*** , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.